MUTABLE LOCI IN MAIZE

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During the past year the study of mutable loci in maize has been continued, in an effort to determine the mode of origin of mutable loci from normal loci and to ascertain the events occurring at a mutable locus that result in detectable changes in phenotypic expression. Progress has been made with respect to both these objectives.

As stated in previous reports, two main classes of mutable loci have appeared and are continuing to appear in the maize cultures. One class includes a number of mutable loci that undergo changes in action only when a second locus, the activator (*Ac*), is likewise present. Mutable loci of the second class do not require such an activator locus. During the past year, study has been continued only on the *Ac*-controlled mutable loci. The decision to confine efforts to these mutable loci was made because all of them respond to the same *Ac* locus, regardless of the diversities of phenotypic expression they represent. On the basis of this common response to the presence of *Ac,* it could be suspected that the events leading to a change in phenotypic expression are of the same nature in all the *Ac*-controlled mutable loci. What are these events? Also, why do normal, "wild-type" loci suddenly become unstable in these cultures?

Previous reports have discussed in detail the *Ac*-controlled mutable *Ds* (dissociation) locus. It was shown that *Ac* may induce chromatid breaks at the *Ds* locus that are followed by fusions of broken ends, and that these fusions may result in the formation of a dicentric chromatid and a U-shaped acentric fragment. It was also pointed out that each such event is comparable, with respect to time and frequency of occurrence, to mutations of other loci that produce recognizable phenotypic changes in genic action. It was concluded that *Ac* must give rise to a specific condition in certain cells of the plant that brings about an alteration in the mode of reproduction of the *Ds* locus in these cells during the mitotic cycle. This alteration eventuates in the production of breaks in the sister chromatids at the *Ds* position, as previously described. By genetical and cytological test methods, it was possible to place this *Ds* locus at a position demarking the proximal third of the short arm of chromosome 9. Continued study, however, has revealed a type of event involving the *Ds* locus that appears to be responsible

for the origin and subsequent behavior of all *Ac*-controlled mutable loci. This event brings about a transposition of the *Ds* locus from one location in the chromosome complement to another. In its new position, *Ds* responds to *Ac* just as it did in its previous position. (The position of *Ds* in the short arm of chromosome 9, where it was first detected, has been designated the "standard position.") These transpositions of *Ds* are not infrequent. In the sporophytic tissues, they usually occur late in development and in individual cells of the plant. For transposition to occur, *Ac* must likewise be present. When *Ds* is transposed from its standard position to another position in the short arm of chromosome 9, the new location may be readily determined.

The Mechanism of Transposition of the *Ds* Locus

A number of cases of transposition of *Ds* are now under investigation. In some of these, a gross chromosomal alteration has accompanied the transposition of *Ds*. By cytological and genetical analyses of the cases involving gross chromosomal aberrations, it has been possible to reconstruct in considerable detail the events that must have occurred to bring about a transposition of the *Ds* locus. These events are similar in all analyzed cases, and can be summarized as follows: During a mitotic cycle a condition may be produced at the *Ds* locus that results in the removal from one or both chromatids of a submicroscopic fragment of chromatin containing the *Ds* locus. Both ends of this fragment are unsaturated; and the mechanism of removal of the fragment may be a tearing process, since unsaturated ends, capable of fusion, are produced in each of the chromatids of chromosome 9 at the position where the fragment was situated. If, during the same mitotic cycle, a spontaneous break occurs elsewhere in the chromosome complement, four additional broken ends may be present in the nucleus. Since any unsaturated broken end is capable of fusion with any other unsaturated broken end, a number of different consequences of fusion among the twelve broken ends can arise. If the spontaneous break occurs in the short arm of chromosome 9 at a position other than the *Ds* locus, several types of altered chromosomes 9 can be formed. These may have a deficiency, a duplication of a segment of the short arm—either in a normal or in an inverted order—or an inversion. On the other hand, fusions of broken ends can bring about a transposition of the *Ds* locus without an accompanying gross chromosomal rearrangement. If the spontaneous break occurs in one of the other chromosomes of the complement, a translocation between the short arm of chromosome 9, at the position of the *Ds* locus, and this other chromosome can be produced. A transposition of *Ds* may likewise accompany such an event. Examples of these various kinds of translocation and transposition have been found. Those involving transpositions of *Ds* within the short arm of chromosome 9, either accompanied or unaccompanied by gross chromosomal rearrangements, have been selected for continued investigation.

In the analyzed cases of transposition of *Ds*, the inserted segment of chromatin containing the *Ds* locus is not visible in its new position with the light microscope. It is also too small to affect detectably the percentage of crossing over in adjacent regions in plants heterozygous for the transposed *Ds* locus. Its detection in the new position is easy, nevertheless, because it behaves as it did in its former position; dicentric chromatids and acentric fragments may be produced by subsequent

breaks and fusions that now occur at this new position. Because it behaves in its new position as it did in its former position, transposition from this new position to still another position may occur subsequently.

The discovery of the transposition of the *Ds* locus, and the knowledge gained in determining the principal events responsible for it, have supplied the information needed for understanding the origin of other *Ac*-controlled mutable loci. It has also become possible to formulate a more direct approach for investigation of the primary effect of *Ac* on the *Ds* locus, wherever it may be, and to determine more fully the various changes that are known to occur at the *Ds* locus itself.

The Origin of *Ac*-Controlled Mutable Loci

In Year Book No. 47 (1947–1948), the sudden appearance of an *Ac*-controlled mutable *c* locus was described. It was found in a single one of the tested male gametes produced by a plant having one *Ac* locus. This plant was also homozygous for a normal *C* locus and for *Ds* in its standard position. In this gamete, the action of the *C* locus had changed. It behaved thereafter like the known recessive (*c*) but, unlike this recessive, was capable of mutating back to a normal *C* action when *Ac* was present.

Study of the c^{m-1} locus has been of particular importance in revealing the factors associated with the origin and subsequent behavior of *Ac*-controlled mutable loci. It is now apparent that the mutable *c* locus arose when the *Ds* locus was transposed from its standard position to a position within or close to the normal *C* locus. This event occurred late in the development of the parent plant, and probably only in a single cell of this plant. No gross chromosomal rearrangements accompanied the transposition. The chromosome 9 carrying this transposed *Ds* locus is morphologically normal. The transposition of *Ds* was recognized by the altered position of the chromatid breaks associated with *Ds* behavior and the concomitant disappearance of such events at the standard location. Both cytological and genetical test methods, used to determine the location of these breaks, were in agreement in placing the *Ds*-type activity at the known position of the normal *C* locus in the short arm of chromosome 9. In its new position, the *Ds* locus presumably inhibits the normal action of the *C* locus. The *C* locus, although present, does not appear to function, and as a consequence no aleurone color is produced. With respect to pigment formation, the tissue response is the same as that given by the known recessive allele, *c*, or by a deficiency of the *C* locus. This inhibited *C* locus, however, can mutate to a state that re-establishes its former action. This occurs only when *Ac* is also present in the nucleus. The restoration may be permanent. The restored *C* locus no longer shows unstable behavior in the presence of *Ac*, and it cannot thereafter be distinguished from a normal *C* locus. What occurs, then, at the inhibited *C* locus to restore its normal action?

As stated previously, the studies of a number of different transpositions of the *Ds* locus have shown that *Ds* may be removed from a chromatid and that the mechanism of removal involves compound chromatid breakage at this locus. The removed fragment containing the *Ds* locus has unsaturated broken ends, and the ends formed in the chromatid by its removal are also unsaturated and capable of fusion. It is known that *Ds* activity usually disappears completely at the c^{m-1} locus when a mutation from *c* to *C* occurs. The known mechanism of removal of *Ds* from a

chromatid, gained from a study of transpositions of *Ds*, suggests an explanation of these mutations. An event leading to removal of the inserted *Ds* segment from the *C* locus would give rise to two broken ends in the chromatid. Fusion of these broken ends would re-establish the former normal genic order, and remove the inhibitory action on the *C* locus induced by the inserted segment; and as a consequence a mutation from *c* to *C* would be evident. No further changes at this locus would occur, for no *Ds* locus would be present to produce them. The *C* locus would be completely normal again. If this primary event is responsible for the *c* to *C* mutations, it also explains why a few of these mutations are accompanied by detectable transpositions of *Ds*. Transpositions could take place if a spontaneous chromosome break, elsewhere in the chromosome complement, occurred in the same mitosis that removed *Ds* from the *C* locus.

The analysis of the events occurring when *Ds* is inserted into or close to the normal *C* locus has made it possible to interpret a previously puzzling aspect of *Ds* behavior at its standard location. At this position, two contrasting "states" of the *Ds* locus have long been recognized. When one of these states (state I) is in effect, the majority of mutational events occurring at the *Ds* locus result in the formation of a dicentric chromatid and a U-shaped acentric fragment. In the contrasting state (state II), there is a markedly lower frequency at this locus of breaks and fusions resulting in the formation of dicentric chromatids or other gross chromosomal rearrangements.

The above two contrasting states of *Ds* may be recognized when it is at the *C* locus (c^{m-1}). In the original isolate of c^{m-1}, a state I *Ds* locus was present. This was the same state of *Ds* that had been present in the chromosome before its transposition to the *C* locus. In kernels having this state of *Ds*, only a few mutations giving a *C* phenotype appear. This state of the *Ds* locus at c^{m-1} changes rather frequently, and by a single event, to one that is comparable to state II of the *Ds* locus at its standard position. The event is made evident by a greatly lowered frequency of dicentric chromatid formation. The rate of *c* to *C* mutations rises to a frequency that is comparable to the previous rate of dicentric chromatid formation. It has been determined that the *c* to *C* mutations are associated with a simultaneous loss of *Ds* activity. This relationship indicates that the change from a *c* to a *C* phenotype is associated with an event involving the *Ds* locus itself. A normal chromosome 9 having a fully active *C* locus but no *Ds* locus is the usual consequence. An interpretation of the event leading to a *C* phenotype has been given above. On this interpretation, the two contrasting states of the *Ds* locus reflect the relative frequencies of alternate consequences of the breakage events occurring at this locus. Both types of consequence are recognized when *Ds* is at the c^{m-1} locus, but only those giving dicentric chromatids or other gross chromosomal abnormalities are detectable when *Ds* is at its standard position. At this latter position, *Ds* may inhibit the action of the adjacent loci, but the inhibition may not be recognized because it results in no obvious change in a readily detectable phenotypic character. In this case, neither the inhibition of genic action brought about by the insertion of the *Ds* locus nor the release from inhibition following its removal would be evident. Detection of the frequency of breakage events at the *Ds* locus would be confined to the fraction that results in the formation of a dicentric chromatid and a U-shaped acentric fragment. This fraction may be high or low, depending on the state of the *Ds* locus.

That the time and frequency of aberrant events occurring at the *Ds* locus may be the same for each of these contrasting states will be indicated in a later section. The important difference is in the consequences of the breakage events, not in the frequencies of the events themselves.

The recognition of different states of the *Ds* locus makes it necessary to consider the factors responsible for the origin of these states and the conditions present in each. Two clearly distinguishable states of *Ds* have been described above. Other states of this locus have been recognized. When *Ds* is at the *C* locus (c^{m-1}), these several states are distinguishable, one from another, by the relative frequencies of the two main consequences of events occurring at *Ds*—that is, dicentric chromatid formation or *c* to *C* mutations. At the standard position, the comparable states are distinguished, one from another, by the relative frequency of only one of these consequences—dicentric chromatid formation. These states appear to be intermediates between the extreme state I and the extreme state II. It has been well demonstrated that a *Ds* locus giving a high frequency of dicentric chromatid formation may change at a single mitosis to one that gives a low frequency. A *Ds* locus giving a low frequency of dicentric chromatids, on the other hand, does not change to one giving a high frequency at a single mitosis. This change from extreme state II to extreme state I requires several stepwise events, reflected in the intermediate states. These observations would suggest that the individual states of the *Ds* locus are indications of the number of active *Ds* units that may be present in a small chromatin segment, and that the change from one state to another involves a change in number and/or distribution of these units within the segment. Such changes might be expected to occur as one of the consequences of the chromatid-breakage-and-fusion mechanism associated with the aberrant events occurring at the *Ds* locus. On this interpretation, it could be concluded that the extreme state II *Ds* locus has few *Ds* units and that the extreme state I *Ds* locus has many such units; for the mechanism could readily reduce the number of units through losses at a single aberrant mitosis, but would require a series of such mitoses to build up a large number of units.

The analysis of the origin and subsequent behavior of *Ds* at the *C* locus has served to clarify some other aspects of this study of mutable loci. Why did new *Ac*-controlled mutable loci arise in these stocks? Why did a normal "wild-type" locus suddenly behave as a mutable locus? What event occurred at the locus to bring about a mutation, that is, a change in phenotypic expression? The analysis of the origin and behavior of c^{m-1} has made it possible to approach these questions and to formulate a concise interpretation of the origin and behavior of the other *Ac*-controlled mutable loci. Inhibition of a locus, either qualitatively or quantitatively, by insertion of a foreign bit of chromatin can be followed by release of this inhibition if the foreign chromatin is removed, transposed, or in some manner altered in position with respect to the inhibited locus. The primary mechanism that allows for such changes at a locus is associated with compound chromatid breaks at the locus and subsequent fusions of the broken ends. In its initial aspects, it is only necessary to consider a single locus having the peculiar faculty of undergoing such breakage events, at whatever position it may be located, to account for the origin and behavior of many different mutable loci.

Transposition of the *Ac* Locus

During the past year, an extensive study of the inheritance behavior of the *Ac* locus

was undertaken. This study has established that *Ac* is inherited as a single unit. It shows typical Mendelian inheritance, with one important exception. This exceptional type of inheritance behavior is the same as that shown by *Ds:* transposition of the locus from one position in the chromosomal complement to another. Two or three per cent of the gametes of an *Ac Ac* plant may be derived from cells in which a transposition of *Ac* has taken place. These transpositions usually occur relatively late in the development of the plant. Plants derived from zygotes that have *Ac* loci in allelic positions in each of two homologous chromosomes may give rise to a few gametes with either (1) two *Ac* loci showing no linkage with one another, (2) two *Ac* loci completely linked or very closely linked, or (3) no *Ac* locus at all. When an *Ac* locus is transposed to a new position, it shows typical Mendelian inheritance at this new position. Linkage with known genic markers can be established. Here, again, exceptions may arise as the consequence of a few transpositions from this new position to still another position. The frequency of these transpositions is not high enough, however, to distort seriously the statistical data of linkage studies. It is likely that the mechanism producing transpositions of *Ac* is the same as or quite similar to that producing transpositions of *Ds*.

Ac itself is a mutable locus. It can be identified only by its action on *Ds*. Its mutations are made evident by changes in the time and frequency of *Ds* mutations. (The events at the *Ds* locus that result in either dicentric chromatid formation or a change in phenotypic expression of a *Ds*-inhibited locus will be termed "*Ds* mutations" in this account.) It is known that the number of *Ac* loci in the nucleus controls the time and frequency of *Ds* mutations. Increased doses of *Ac* loci (from 1 to 3 in the triploid endosperm) result in an increasingly delayed time of occurrence of *Ds* mutations. Similar changes in the mutational response of *Ds* will be registered after a somatic mutation in a single *Ac* locus. These responses indicate that some quantitative change may take place at the *Ac* locus when it mutates—probably an increase or decrease in the number of subunits at this locus. Thus, superimposed on those quantitative changes that can be produced by additions of whole *Ac* loci through controlled chromosome combinations in diploid tissues of the plant or in triploid tissues of the endosperm are those that can occur at a single *Ac* locus.

There is a ready method of identifying those kernels on the ears of *Ac Ac* plants that are likely to have a transposed *Ac* locus. This involves crossing plants having no *Ac* locus to plants having a single *Ac* locus in which the *Ac* state is known (determined by its effects on *Ds* in 1, 2, and 3 doses). The F_1 plants are selfed and the F_2 progeny grown. The F_2 plants are then crossed by plants having no *Ac* locus but carrying *Ds* at its standard location in each chromosome 9. The ears produced by the *Ac Ac* F_2 plants are selected, and an examination is made of the *Ds* mutation rates in the kernels. If, in the *Ac Ac* F_2 plants, no mutations have occurred at the *Ac* locus and no transpositions have taken place, all the kernels should show the same pattern of *Ds* mutations. In other words, the control of these *Ds* mutations should be the same, since all the kernels should have two *Ac* loci in the endosperm cells and all the *Ac* loci should be alike. The majority of the kernels on such ears do show a remarkable similarity in the pattern of expression of *Ds* mutations. A small percentage of the kernels, however, are markedly different. These exceptional kernels fall into three classes: (1) those showing no *Ds* mutations at all,

(2) those showing a few very late-occurring *Ds* mutations that suggest an increase in *Ac* dosage, and (3) those showing a time and frequency of *Ds* mutations that suggest a lowered dosage of *Ac*. A preliminary test was made in an attempt to determine the reason for the changed responses of *Ds* in the kernels of types (1) and (2). Twenty-five such kernels were selected from these ears, and plants were grown from them. Tests were conducted to determine (1) the presence or absence of *Ds*, (2) the presence or absence of *Ac*, and (3) the action of *Ac*, when present, in one and two doses. Eleven of the plants arising from these selected kernels gave no evidence of *Ac* at all; the *Ac* locus was either absent altogether or completely inactive. Ten other plants had two independent, nonlinked *Ac* loci. In four plants, *Ac* was inherited as a single unit; but this unit, in a single dose, produced the same effect on *Ds* mutations that two units of the original *Ac* locus, from which it was derived, had produced.

One type of event, the transposition of *Ac*, will account for these results. If, in these *Ac Ac* F_2 plants, transposition of one of the *Ac* loci occurred in a meiotic or premeiotic mitosis, two *Ac* loci would still be present in the nucleus, but they would no longer be allelic with respect to position in the chromosomal complement. If the transposed *Ac* locus were inserted into a nonhomologous chromosome, meiotic segregations could give rise to gametes with either (1) one *Ac* locus, in its original position or its new position, (2) two *Ac* loci, one in each of two nonhomologous chromosomes, or (3) no *Ac* locus. Transposition within the same chromosome (or homologue), or insertion of the *Ac* locus of one chromatid adjacent to the *Ac* locus of the sister chromatid, would give comparable meiotic segregations with respect to the production of gametes with two *Ac* loci or with no *Ac* locus.

In the given cross, the kernels arising from the megaspores having no *Ac* locus would show no *Ds* activity; for no *Ds* mutations occur without *Ac*. Tests for *Ac* in the plants arising from these kernels would be negative, because no *Ac* locus would be present. Kernels developing from megaspores receiving a single *Ac* locus, either in its original position or transposed but unmodified in its action, would show the characteristic effect on *Ds* mutations produced by the *Ac* locus when two are present in the endosperm. (It should be recalled that the female parent contributes two nuclei to the triploid endosperm tissue, and the male parent one.) Those developing from megaspores with two *Ac* loci, either linked or situated in different chromosomes, would give rise to endosperms with four instead of two *Ac* loci. It is known that increases in the dose of *Ac* will delay the time of appearance of *Ds* mutations, and that this effect is proportional to dosage—the higher the dose, the more effective the delay. With four doses of *Ac* instead of the usual two, the delay may be so effective that either no *Ds* mutations will occur during the development of the tissue or only a few will occur very late in the development of the endosperm. In either case, the kernels having such increased doses of *Ac* will be strikingly different in appearance from the majority of kernels, that is, those with two *Ac* loci in their endosperm cells. It was this striking difference in appearance of a few kernels on these ears that allowed the selection to be made. The analysis of the *Ac* composition of the kernels has led to the conclusion that they develop from ancestor cells in which a transposition of *Ac* has occurred.

For comparison, plants were grown from some of the kernels on these F_2 ears that

showed the characteristic type of *Ds* mutational response known to be associated with the presence in the endosperm of two *Ac* loci. Tests of the *Ac* constitution of these plants gave the expected results. One *Ac* locus was present in each of the tested plants, and its control of the time and frequency of *Ds* mutations, in one or two doses, was similar to that in the parent plant.

These studies have been expanded during the summer of 1949; but the results of the preliminary tests are sufficient to indicate the factors responsible for apparent exceptions to the expected Mendelian inheritance of *Ac*. They have also made possible an interpretation of one of the several kinds of event that occur during the development of the plant or of the endosperm to bring about pronounced changes in the action of *Ac* on *Ds*. These changes are registered by the appearance of precise sectors showing altered *Ds* responses. Tests are now being conducted to distinguish between changes in state of the *Ac* locus—that is, between changes in quantitative action of an *Ac* locus that is inherited as a single unit, and changes that are caused by an increase in numbers of such loci after transposition of *Ac*, as outlined above. The phenotypic effects of these two types of change overlap, but the causative series of events, although related, are nevertheless separable.

The mechanism responsible for transposition of the *Ac* locus has not been analyzed. It is thought likely to be the same as or similar to that producing transpositions of *Ds*. If so, some of the transpositions of *Ac* should be associated with chromosomal rearrangements. A chromosomal translocation was recognized in one of the cases cited, but it has not yet received adequate analysis.

The Action of *Ac* on the Mutable Loci It Controls

It has been emphasized repeatedly that *Ac* controls the occurrence of *Ds* mutations and that its quantitative levels control the time and frequency of these mutations. In this report, it has been shown that the mutable c^{m-1} locus is merely a transposed *Ds* locus situated at or close to the *C* locus. The analysis of this c^{m-1} locus and of its origin from a transposition of *Ds* has suggested that all *Ac*-controlled mutable loci arise from transpositions involving, originally, only one *Ds* locus. According to this interpretation, *Ac* does not control the mutability of many different loci, but only the mutability of a single locus—the *Ds* locus—wherever it may be situated in the chromosomal complement. Mutations of *Ds* in these various positions may result in changes in phenotypic expression that are strikingly different. The change in phenotype, in any one case, depends on the kind of locus that has been inhibited by the insertion of *Ds*. In their normal action, these various *Ds*-inhibited loci must control quite different chemical processes. The events at the *Ds* locus that result in a return to partial or complete action of the inhibited locus must therefore involve a different series of changes in chemical processes in each case. Without an integrative understanding of the events that occur at such mutable loci, it would be difficult to understand why *Ac* should control the mutability of loci concerned with such unrelated processes, and why each such locus should respond to a particular *Ac* locus and dosage in an exactly comparable manner. There is no difficulty, on the basis of the given interpretation, in appreciating the apparent nonselectivity of control of mutable loci by *Ac* and the similarity in response of these mutable loci to changes in *Ac* state and dosage.

In order to obtain more specific information about the nature of the action of *Ac* (other than its known effects in producing chromatid breaks at the *Ds* locus and controlling the time and frequency of these breaks), combinations of *Ds* loci at various positions in the short arm of chromosome 9 have been made. These combinations were made in an attempt to answer the following question: Does *Ac* produce a cellular or nuclear condition in a certain cell, at a certain time in development, to which all *Ds* loci will respond? An instructive example for this purpose is a combination of c^{m-1} (*Ds* at or close to the *C* locus) with *Ds* at its standard location. If a plant carrying c^{m-1} and *wx* in its chromosomes 9 is crossed by a plant carrying c^{s} (stable *c*, nonmutable with *Ac*), *Wx*, and *Ds* (standard location, to the right of *Wx*), kernels will be produced that are c^{m-1} *wx*/c^{m-1} *wx*/c^{s} *Wx Ds*. This combination should show whether or not mutations in the several *Ds* loci will occur at the same time in the same cell, and whether this response will be of the same order with one and with more doses of *Ac*. Simultaneous mutations would be revealed in these kernels provided an extreme state II *Ds* locus were present at c^{m-1} (mutation from *c* to *C* and few if any dicentric chromatid formations), and an extreme state I *Ds* locus were present in the c^{s} *Wx Ds* chromosome (high rate of dicentric chromatid formation). If all *Ds* loci respond to some particular developmental change that is brought into being by the presence of *Ac*, then when this changed condition arises in a cell, a mutation of *Ds* at the c^{m-1} locus should give a *C* phenotype in the descendent cells. A mutation at the *Ds* locus in the c^{s} *Wx Ds* chromosome should also occur. A *wx* phenotype would then appear in the descendent cells, because a *Ds* mutation in the c^{s} *Wx Ds* would produce a dicentric chromatid and a U-shaped acentric fragment; this acentric fragment would carry the *Wx* locus, and consequently *Wx* would be lost from the nuclei during a mitosis. The effects produced by such simultaneous mutations of the several *Ds* loci should be visible in the mature kernel. Colored areas (the *c* to *C* mutations) should appear, and the underlying starch should be *wx*. Also, the borders of the sectors having both of these altered phenotypes should correspond exactly. In the examined kernels having these given constitutions, a high percentage of the *C* areas had underlying *wx* starch, and the borders of the sectors did exactly correspond. Exceptions were expected, and a number were observed. Some examples were: *C* areas with underlying *Wx* starch, *wx* areas with overlying colorless aleurone, *C* areas with only half of the underlying sector composed of *wx* starch, or *wx* areas with only half of the overlying aleurone layer showing a *C* phenotype. It is hoped that an extended analysis of the various classes of exceptional areas in these kernels will reveal the more unusual consequences of the events that occur at the *Ds* loci in these mutation-producing mitoses, and the resultant organization in the two affected sister chromatids.

Tests have also been constructed to determine the relation between the mutations of *Ac* and those of *Ds*. Although the analyses of these tests are incomplete, it seems apparent that *Ac* tends to mutate in the same cell in which a *Ds* mutation is occurring, or in an immediate ancestor cell. The combined evidence suggests that some condition, under the control of the *Ac* locus and depending on its state and dosage, must develop in specific cells at specific times, to produce a mutational response (chromatid breaks) at *Ds* loci as well as at the *Ac* locus itself. The consequences of such mutation are the observed changes in

genic action, transpositions or losses of *Ds* or *Ac*, and production of gross chromosomal rearrangements with or without accompanying transpositions of *Ds* or *Ac*.

Mutable Loci c^{m-2} and wx^{m-1}

The *Ac*-controlled mutable loci c^{m-2} and wx^{m-1} were described in Year Book No. 47. A few salient facts and conclusions based on the continued study of these loci are as follows: Both loci express their mutations quantitatively. A series of alleles derived from such mutations, which show gradations of quantitative expression, have been selected for study. When *Ac* is absent, a particular expression of an allele can be held constant, for no somatic mutations of these alleles occur. When *Ac* is present, the alleles may continue to mutate to either higher or lower levels of quantitative expression. For a study of the action of any one allele, therefore, it is important that no *Ac* locus be present.

It has been determined that chromatid breaks may occur at these two mutable loci; in this respect, they are similar to c^{m-1}. Both c^{m-2} and wx^{m-1} were isolated from stocks known to have a *Ds* and an *Ac* locus. Unlike c^{m-1}, they were not detected at the time of their origin. It is therefore impossible to reconstruct the particular events associated with their origin from a normal *C* locus and a normal *Wx* locus. The presence of *Ds*-type behavior at these mutable loci points to a mechanism similar to the one associated with the origin of c^{m-1}.

In the case of c^{m-2}, the position of insertion of *Ds* into or adjacent to the *C* locus may differ from its position of insertion in c^{m-1}; for two qualitatively different types of phenotypic expression of the *C* locus result from mutations of c^{m-2}, whereas only one type regularly follows mutations of c^{m-1}. Both types of qualitatively distinguishable mutations at c^{m-2} result in pigment formation in the aleurone layer. Within each of the two qualitative types there occurs a series of mutants showing various degrees of quantitative expression. The color intensities produced by the different mutants of both types range from a faint pink to a deep red (in *pr pr* constitutions). The two series of mutants are distinguished from each other mainly by the fact that a different diffusible substance (or substances) is produced by the members of each. Both substances are concerned with pigment formation. The diffusible substance produced by type 1 mutants may be utilized by a cell having a normal *C* locus, or by a cell having a type 2 mutant, to intensify the color of the cell pigment. The normal *C* locus and the type 2 mutants, on the other hand, both produce a diffusible substance that can be used by type 1 mutants to intensify pigment color. Thus, the type 2 mutants and the normal *C* locus are much alike; they both produce a diffusible substance that type 1 mutants can use, and they both can use a diffusible substance produced by type 1. This relationship suggests that a normal *C* locus is probably responsible for the production of at least two diffusible substances, both of which are required for pigment formation. It also suggests that the dosage responses noted for the normal *C* locus may be the consequence of a limited production of one of these substances by a single *C* locus: the more *C* loci were present, the more of this substance would be produced and the deeper would be the pigment color. The quantitative grades of expression of the alleles within the two types of mutations arising from c^{m-2} may reflect the relative quantities of the two substances produced by individual members of a type—limitations in the production of one of these substances conditioning the amount of pig-

ment that can be formed, and thus the depth of color that can appear.

The conclusions derived from study of c^{m-2} regarding the action of the normal *C* locus are noteworthy, in that they consider a double function of a single unit in inheritance. This unit, concerned with pigment production in the aleurone layer of the endosperm, appears to be composed of at least two qualitatively different subunits, both of which determine the production of substances required for pigment formation. It is possible that this *C* locus behaves as a unit in inheritance not only because all the subunits are needed for the production of pigment, but also because a particular spatial relation of the units at the locus is required to assure a definite sequence of reactions.

Mutations of the wx^{m-1} locus have been similarly instructive in considering the action of the normal *Wx* locus, but for reasons other than those just discussed for c^{m-2}. Here, alleles showing various quantitative levels of expression are produced by mutations of wx^{m-1}. The levels are expressed by the percentage of amylose in the starch component of the endosperm cells. When only the recessive, *wx*, is present, no recognizable amylose starch is produced. The selected alleles derived from mutations of wx^{m-1} form a series in which a single dose (*Wx* allele, *wx*, *wx*) produces quantities of amylose ranging from very little (less than 1 per cent) to as much as the normal *Wx* locus produces in three doses. Chemical analyses of the percentages of amylose starch produced by several of these alleles have been conducted by Miss Ruth Sager and Dr. Charles O. Beckmann, of Columbia University. These analyses have shown that the type of color reaction produced by staining with iodine is a relatively reliable indication of the approximate percentage of amylose present.

Interest in this case centers not so much in the appearance of alleles having lower activity than the normal *Wx* locus as in those having higher activity than the normal locus. Is the normal *Wx* locus partially inhibited, or do the *Wx* alleles showing greater than normal activity arise from duplications of the locus? In *Ac*-carrying plants, the chromatid-breakage-and-fusion mechanism associated with mutations at the wx^{m-1} locus or its intermediate alleles should give rise, in some cases, to duplications or multiplications of units of the *Wx* locus. It is hoped that a study of the different amounts of amylose produced by sister chromatids after mutation of wx^{m-1}, or one of the intermediate alleles, will furnish some information with reference to this question.

Conclusions

The purpose of the foregoing sections has been to indicate the progress made during the past year in attacking fundamental aspects of the origin and behavior of *Ac*-controlled mutable loci. It was concluded that only two loci are involved in all these cases: the *Ds* locus and the *Ac* locus. The origin and subsequent behavior of newly arising mutable loci depends on the transposition of a *Ds* locus and its insertion into (or adjacent to) a normal locus, and on the constitution of this inserted *Ds* locus. The genic action of a normal locus may be inhibited by such an insertion. Subsequent events at this new position may remove the inserted segment and its inhibitory action altogether; or changes in the constitution or position of the *Ds* locus may result in changes in the degree of inhibition of the affected locus. It was also concluded that the events occurring at *Ds* during a mutation-producing mitotic cycle result in compound chromatid breaks at this locus, and that the observed consequences depend on subsequent fusions of the broken ends.

The fusion phenomenon, of utmost importance in these cases, calls for no new interpretations, since the fusion of newly broken (unsaturated) chromosome ends has been well investigated and could be anticipated.

Both *Ac* and *Ds* are mutable loci, for their mode of action changes as the consequence of events occurring at these loci in certain cells of the plant. Like *Ds, Ac* also undergoes transposition from one location in the chromosomal complement to another. The mechanism of transposition, although not directly analyzed, is possibly similar to that associated with the transpositions of *Ds*. The evidence also indicates that changes in *Ac* as well as *Ds* are associated with chromatid breakage and fusion. It is necessary to determine, then, the nature of the events occurring at these two mutable loci, during a particular mitotic cycle, that will result in the observed breakage-and-fusion phenomena. Unquestionably, these events are primarily responsible for all the observed changes at these mutable loci. It is suspected that they are associated with some aberration in the mode of reproduction of a particular type of molecule in the chromosome during a mutation-producing mitotic cycle. Both *Ac* and *Ds* are assumed to have such molecules. If the aberration involves a chemical bonding of the newly formed molecule with the original molecule, which holds at least until after the forced separation of sister chromatids during the prophase period, a rupture of the chromatid could occur at the affected locus during this separation period. It is known that the bonds holding the molecules together in a linear order in the chromosome may be ruptured by mechanical pull, and that the broken ends so produced are unsaturated and capable of fusion with other unsaturated broken ends. It is therefore necessary to assume, in this interpretation, that the bond connecting the newly formed molecule with the original molecule is stronger than the bond holding the molecules together in linear order.

The study of transpositions of the *Ds* locus has shown that a rupturing mechanism of this type, or at least one that leads to similar consequences, must be involved. It has been established that the transposition phenomenon is associated with chromatid breakage; the *Ds* locus is inserted into a position where a spontaneous break has occurred. The transposition phenomenon is readily explained if it is assumed that break-producing events at the *Ds* locus may sometimes result in the tearing-out of a minute fragment containing *Ds* and having two unsaturated broken ends. The insertion of *Ds* into a new position would result merely from fusion of unsaturated ends. If the broken ends arising from the spontaneous break are labeled 1 and 2 and those of the fragment 3 and 4, the fusion of 1 with 3 and 2 with 4 would accomplish the transposition. The above-described process of mechanical rupture of the chromatid at the *Ds* position could result in just such a torn-out fragment. The consequence of any one rupture would depend on the type of fusion of broken ends that followed. Not only could transpositions occur, but the *Ds* locus could be lost altogether, or two *Ds* loci could enter one chromatid, leaving none in the sister chromatid. Such duplications (altered states of *Ds*) could, in turn, initiate a series of new consequences when the aberrant type of event, leading to chromatid rupture, again occurred in a descendant of this chromatid.

The analysis discussed in this report of the factors associated with the origin and behavior of *Ac*-controlled mutable loci in maize has led to a relatively simple interpretation of the nature of the events responsible for changes in action of the genes

involved. The types of phenotypic change that follow mutations of non-*Ac*-controlled mutable loci are similar to those shown by the *Ac*-controlled mutable loci. It is quite possible that the same or similar events are primarily responsible for these changed phenotypes also.

Mutable loci have been described in a number of organisms. Many of them show changes in phenotypic expression similar to those now being observed in maize. The events responsible for changes in expression of genic action may be similar in these organisms to those occurring in maize. The investigations described in this report cast doubt on interpretations that postulate a "true gene mutation," that is, a chemical change in a gene molecule, resulting in a changed specificity of its active product. Phenotypic change may well be related to inhibition of the action of a normal gene followed by partial or total release of this inhibition, together with such duplications or deficiencies of the locus as could be produced by the mechanism outlined above.